25. A plant or its progeny regenerated from a plant cell of claim 16 containing and expressing said gene in insecticidal amounts.

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REMARKS

The Examiner is thanked for discussing this application with the undersigned on February 22, 1989. In accordance with that discussion, the claims hereof have been amended to cancel the vector claims, to specify the use of the Bacillus thuringiensis crystal protein insecticide gene, to specify that said gene is expressed in insecticidal amounts in plant tissue and plants, and to specify that the plant cell being transformed is one which is capable of regeneration.

Also in accordance with that discussion, the parent application, serial no. 848,733 is being expressly abandoned and the outstanding rejections therein are discussed below.

The Amendments

Claims 1-14 have been cancelled. Claims 16-25 have been added specifying regenerable plant cells transformed with the Bacillus thuringiensis crystal protein insecticide gene such that the plants and plant tissue regenerated therefrom are insecticidal. Claims specifying both full-length and truncated genes have been added. Support for these amendments are found in the original claims, the paragraph bridging pages 2 and 3, the paragraph bridging pages 37 and 38, the paragraph bridging pages 38 and 39, the paragraph bridging pages 58-60, and the Examples.

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The Invention

This specification represents the first demonstration of successful expression of Bacillus thuringiensis crystal protein insecticide genes in plants such that insects who naturally feed on the plants will be killed. Surprisingly, it was found that the expression of this toxic protein in plant cells did not prevent regeneration of plant tissue and whole plants expressing the gene, or kill the plant cells. Also, surprisingly, it was found that the gene could be expressed in amounts sufficient to kill the attacking insects.

Applicants, with the filing of the present continuation-in-part application disclose the actual reduction to practice of the invention with a number of important crop plant species, both monocots and dicots.

The Rejections in the Parent Application

Scope

The Office Action issued in the parent application, serial no. 848,733, on January 19, 1989 rejected the claims under Section 112 stating that they were enabled only for tobacco plants transformed with a full-length Bacillus thuringiensis HD-73 gene.

This amendment narrows the claims to specify Bacillus thuringiensis genes. As was discussed with the Examiner in the interview on February 22, 1989, the present continuation-in-part application exemplifies a number of different plants and a number of different Bacillus thuringiensis insecticidal genes. A listing of some of the genes and plants exemplified is provided below. There is no reason to believe the invention would not be as operable in other plant species with other B.t. genes as it is with those plants and genes exemplified. Applicants believed, in light

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of their knowledge of the mechanisms involved, at the time the grandparent application was filed, that the B.t. gene would be expressible in insecticidal quantities in any chosen plant, and this is what they claimed. Their later work showed the correctness of this position and corroborated their original claims.

This continuation-in-part application contains laboratory data showing expression of the following genes in the following plant materials:

Gene	Plant	Pages
Full-length HD-73	Tobacco	36,115,122
Partial HD-73	Tobacco	75,81,88
Full-length HD-73	Tomato	36,144,147
Full-length HD-73	Cotton	38,160
Full-length HD-73	Potato	159
BTt (partial)	Maize tissue	140

Also, the cited art as well as applicants' later work and the additional art discussed in the attached Declaration of Inventor Michael J. Adang shows the usefulness of B.t. genes from additional strains, thus corroborating applicants' broad enabling statements.

The Examiner cited Barton et al. and Vaeck et al. in support of his contention that it was not predictable that any B.t. gene could be used in any plant. Barton et al. reported that tobacco callus regenerated under kanamycin selection after transformation with the full-length HD-1 B.t. gene under control of a strong promoter died. Vaeck et al. reported in the text that tobacco tissue transformed with the full-length Bt2 gene from strain berliner 1715 under control of a weak promoter did not show insect-killing activity greater than the control NPTII-expressing construct; however, in Table 2, they do show insect-killing activity for this full-length Bt2 construction.

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The rejection using Barton et al. and Vaeck et al. is legally improper since a reference published after an applicant's filing date may not be used to show lack of enablement. In re Hogan, 194 U.S.P.Q. 527 (C.C.P.A. 1977). Barton et al. and Vaeck et al. were both published in 1987. The application to which the rejection was applied was filed in 1986 and claimed priority to the original application filed in 1983. The present application claims priority of both earlier applications.

During the interview on February 22, 1989, the Examiner requested an explanation of the fact that applicants were able to obtain expression of a full-length B.t. gene in insecticidal amounts in tobacco without killing the tobacco tissue while other workers reported either tissue toxicity or no insect toxicity with full-length genes. Since the citation of the later-published references is improper, such an explanation should not be legally necessary, however, applicants wish to satisfy the Examiner's scientific doubts and point out the operability of their invention using the B.t. genes described in Barton et al. and Vaeck et al. and have therefore provided such an explanation in the enclosed Declaration of inventor Michael J. Adang.

Applicants submit that the plant toxicity results reported by Barton et al. and the low kill rate reported by Vaeck et al. were likely caused by failure to optimize parameters for the method of this invention. Such optimizations are well within the ordinary skill in the art once the information in applicants' disclosure is known.

To summarize the Declaration, as disclosed in applicants' specification, the full-length HD-73 gene under control of a fairly weak promoter (the T-DNA ORF 24 promoter) was expressed in low levels (up to 2 ng/mg) in tobacco. These levels were sufficient to kill insects in bioassays, but considerably lower than the

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levels reported by Barton et al. (12 ng/mg) when the full-length HD-1 gene was expressed under control of a strong (CaMV 35S promoter) in tobacco. The Vaeck et al. promoter was the same weak promoter used by applicants. The Vaeck et al. results are comparable or very slightly lower than those achieved by applicants. The lower insect-killing activity achieved by both Vaeck et al. and applicants using the full-length gene with a weak promoter is still useful, all as set forth in the attached Inventor's Declaration.

Applicants cannot explain the inconsistencies in the literature which the Examiner has cited. Any attempt at an explanation is merely speculative. It sometimes happens in the early stages of research in complex systems that workers using different constructs, different test organisms, different media, etc. may fail to achieve optimum results. Such inconsistencies are to be expected. The Cohen-Boyer process itself cannot operate properly without the optimal functioning of all its components: proper reaction conditions for each restriction enzyme, each ligase, and proper host cells that lack incompatible plasmids, etc. etc. etc. All such details are matters of optimization that those of ordinary skill in the art can perform without undue experimentation. In any event, Applicants know of no undisclosed operating parameters that could render the invention as taught and claimed inoperative, other than those well-known in the art (plants must be alive, enzymes must be active, etc.).

The higher levels of toxin expressed by Barton et al. using the strong promoter may explain the apparent toxicity to the plant. The low levels of toxin expressed by Vaeck et al. using the weaker promoter may explain the low toxicity to insects. Adjustment of expression levels so as to prevent plant toxicity while retaining insect toxicity would be a matter of ordinary skill in the art, especially in light of the teachings and data presented by

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applicants herein showing that this optimization can be achieved using a full-length construct.

In general, applicants achieved better results when using the partial genes than when using the full-length genes, and better results in tomato than tobacco. See, e.g., Fischoff, et al. (1987) Biotechnology 5:807-813 and applicants' later data provided in the Adang Declaration. For example, the full-length HD-1 gene found to be toxic by Barton et al. to tobacco when expressed under the CaMV 35S promoter was found by applicants not to be toxic to tomato. It is not to be expected that all constructs would provide identical levels of expression. However, applicants have shown that in general, any B.t. gene can be expressed in insecticidal amounts in any plant provided system parameters are optimized according to principles known in the art, e.g., selection of appropriate promoters, enhancers and the like.

In any event, all of applicants' claims as amended require that the transformed cell be "capable of regeneration." A cell in which B.t. is expressed at levels high enough to kill the cell is, by definition, not capable of regeneration and, therefore, not within the scope of the claims.

Even in the event there might be some plants or some genes in which no insecticidal activity could be obtained by optimization (which has never been reported), or if obtaining expression in a particular instance involved a higher level of skill than that of the ordinary skilled worker, this would in no way invalidate applicants' claims. It is well settled that it is not the function of claims to exclude all inoperative species. In re Dinh-Nguyen et al., 181 U.S.P.Q. 46 (C.C.P.A. 1974), Ex parte Janin 209 U.S.P.Q. 761 (Pat. Off. Bd. Appl. 1979).

The purpose of a specification is not to teach how to make all possible combinations and permutations of an invention. It is to

teach the best mode and to provide clear guidelines to infringers as to whether they are infringing or not. This applicants have done. Applicants were the first to show that B.t. genes could be expressed in plants so as to make them lethal to insects attacking them, and they are entitled to patent protection commensurate in scope with this important discovery.

Obviousness

The Office Action issued in the parent application rejected the claims based on a combination of references which teach the expression of antibiotic resistance genes in plant cells. As applicants have previously argued, expression of such genes in plant cells does not make it obvious that an insecticidal toxin such as B.t. could be expressed in plant tissue so as to render such tissue insecticidal. Insecticides are poisons and might be expected to be lethal to the cells producing them. In fact, as the Examiner points out, Barton et al. disclose that the full-length HD-1 gene expressed under control of a strong promoter is toxic to tobacco cells.

At least four important factors were unknown and could not have been predicted prior to applicants' invention: (a) that B.t. could be expressed in plant cells in amounts that were not lethal to the plant cells; (b) that plant tissues and plants could be regenerated from cells transformed with toxic B.t. proteins; (c) that B.t. could be expressed in plant cells in amounts that would kill insects; and (d) that plants expressing the B.t. gene would actually be toxic to insects wihtout further treatment, e.g., by arranging to have the toxin excreted from the plant cells. The unpredictability of all these factors means that the requisite reasonable predictability of success required for an obviousness rejection is not present in this instance and the claims should be found to be non-obvious over the prior art. In re O'Farrell, 7 U.S.P.Q. 2d 1673) (C.A.F.C. 1988) cited by the Examiner involved

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only one predictable factor, not the several unpredictable factors of the present case.

The Examiner supports his position that success is predictable based on the fact that B.t. is used on crops. Applicants point out, however, that when B.t. is applied topically to plants, it is not placed inside the cell where it might be toxic as in the present invention, and one would not know whether insecticidal amounts of toxin could be present in a transformed cell without killing the cell or whether other intracellular components might bind to the toxin and inactivate it. Applicants showed that indeed plants could be transformed to make them insecticidal.

The Examiner also supports his position that success is predictable by referring to "the primary references' use of antibiotic genes which encode compounds known to be lethal to microbial life forms." However, the primary references disclose antibiotic resistance genes, not antibiotic genes. In many instances, bacteria which produce antibiotic genes also produce corresponding resistance genes without which the bacteria would be killed by the antibiotic (e.g., the Streptomyces bacteria). No B.t. resistance gene had been identified, and it might have been expected that such a gene would have been required along with the B.t. toxin gene in order to avoid toxicity to the host organism. However, applicants showed that such was not the case and that it was possible to achieve viable regenerated plants containing a B.t. toxin gene without a B.t. resistance gene.

Conclusion

In view of the foregoing arguments and amendments, it is submitted the application is in condition for allowance, and passage to issuance is respectfully requested.

It is believed that the present amendment does not require the payment of any additional fees under 37 C.F.R. §1.16-1.17. If this is incorrect however, please charge any fees required under the foregoing rules to deposit account no. 071969.

Should the Examiner wish to maintain any of the rejections discussed above, a telephone interview is respectfully requested and the Examiner is invited to telephone the undersigned to arrange a mutually convenient time.

Respectfully submitted,

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